Name of Organization: Johns Hopkins University School of Hygiene and Public Health

Type of Organization: College or University

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Project Title: Great Lakes zebra mussels and Cryptosporidium contamination

Project Category: Emerging Issues

Rank by Organization (if applicable): 0

Total Funding Requested ($): 166,317 **Project Duration:** 2 Years

Abstract:

GREAT LAKES ZEBRA MUSSELS AS BIOLOGICAL INDICATORS OF WATERBORNE CRYPTOSPORIDIUM CONTAMINATION IN THE VICCINITY OF DRINKING WATERPLANT INLETS

Zebra mussels can recover and concentrate the environmentally-derived human waterborne pathogen, Cryptosporidium parvum, and therefore can be used for sanitary assessment of the quality of raw water drawn for drinking water processes. Cryptosporidium parvum is an intestinal waterborne pathogen that significantly contributes to the mortality of people with impaired immune systems and morbidity of immunocompetent persons due to a lack of effective therapy. The pathogen is frequently transmitted via drinking water as a result of contamination of surface water by urban and agricultural runoff and wastewater discharges. We propose an innovative and creative multi-disciplinary 2-yr project in which we will collect zebra mussels in temporal and spatial fashions from the water inlets (or their vicinities) of drinking water facilities and from sites affected by wastewater discharges and agricultural runoff at the Great Lakes (i.e., Lakes Michigan, Huron, and Erie). The project objectives and outcomes include: 1) Evaluation of the applicability of zebra mussels for biological monitoring of waterborne contamination of Great Lakes with C. parvum; 2) Assessment of the extent of environmental pollution of Great Lakes with C. parvum, and determination of the intensity of pathogen load from urban and industrial (wastewaters) and agricultural (livestock operation areas) sources to the surface water; 3) Assistance to drinking waterplant managers by providing information on the presence of C. parvum oocysts in raw water, and 4) Determination and assessment of the role of zebra mussels in removal of waterborne C. parvum oocysts from the Great Lakes water.

Geographic Areas Affected by the Project

States:

- ☐ Illinois
- ☐ Indiana
- ☒ Michigan
- ☐ Minnesota
- ☐ New York
- ☐ Pennsylvania
- ☒ Wisconsin
- ☒ Ohio

Lakes:

- ☐ Superior
- ☒ Huron
- ☒ Michigan
- ☒ Erie
- ☐ Ontario
- ☐ All Lakes

Geographic Initiatives:

☐ Greater Chicago ☐ NE Ohio ☐ NW Indiana ☐ SE Michigan ☐ Lake St. Clair

Primary Affected Area of Concern: Saginaw River, MI

Other Affected Areas of Concern:

For Habitat Projects Only:

Primary Affected Biodiversity Investment Area: Saginaw Bay

Other Affected Biodiversity Investment Areas:

Problem Statement:

GREAT LAKES ZEBRA MUSSELS AS BIOLOGICAL INDICATORS OF WATERBORNE CRYPTOSPORIDIUM CONTAMINATION IN THE VICCINITY OF DRINKING WATERPLANT INLETS

Cryptosporidium parvum is an intestinal pathogen that significantly contributes to the mortality of individuals with impaired immune systems due to a lack of effective therapy (4). The parasite is frequently transmitted via drinking water that contains the oocysts (parasite infective stages), as a result of contamination of surface water by agricultural and urban runoff and wastewater discharges (4). The largest outbreak of cryptosporidiosis due to drinking water in US history occurred in 1993 in Milwaukee, resulting from contamination of Lake Michigan and some operational deficiencies at waterplants drawing water from this lake (4).

Molluscan shellfish can harbor environmentally-derived oocysts of C. parvum as a result of filtering large water volumes and concentrating the oocysts (1-5). Examination of such shellfish can prove water pollution even when standard water testing yields negative results (1-5). Oysters, mussels, and clams are excellent indicators of contamination of marine waters with C. parvum (1-4). However recently, this pathogen was identified for the first time in freshwater bivalves, zebra mussels, from the St. Lawrence River (5). The zebra mussel is a highly invasive, and the most recently introduced bivalve species to North American freshwaters invading the Great Lakes (5). Major incidences of zebra mussel folding resulting in clogged pipes have been reported in water facilities on Lakes St. Clair, Erie, and Ontario (5).

We propose an innovative and creative, multi-disciplinary 2-yr project in which we will collect zebra mussels in temporal and spatial fashions from the inlets (or their vicinities) of waterplants that draw raw water from Great Lakes, i.e., Lakes Michigan, Huron, and Erie. Mussel samples will be also collected from the vicinity of potential pollution sources, i.e., wastewater discharges and intense agricultural runoff. The main objectives of the project include: A) Evaluation of the applicability of zebra mussels for biological monitoring of waterborne contamination of Great Lakes with C. parvum; B) Assessment of the extent of environmental pollution of Great Lakes with C. parvum, C) Assistance to drinking water facility managers by providing information on the presence of C. parvum oocysts in raw water, and D) Determination and assessment of the role of zebra mussels in removal of waterborne C. parvum oocysts from the Great Lakes. Our objectives are consistent with the missions, goals, and objectives of the USEPA's Great Lakes National Program Office.

Groundbreaking studies on environmental contamination of the Chesapeake Bay, MD, with C. parvum and recovery of the pathogen by commercially and non-commercially harvested oysters have been intensively conducted by Dr. Graczyk at Johns Hopkins University, and by collaborative laboratories at USDA, NOAA, and CDC (1-5). These studies were sponsored by Maryland Sea Grant, R/F-88. As a result, conventional and molecular technologies have been developed for recovery and sensitive/specific detection of viable C. parvum oocysts in shellfish (1-5). These technologies will be applied in the proposed project which has strong community-based support and addresses environmental justice issues.

Literature Cited

1. Fayer R, Graczyk TK, Lewis EJ, Trout JM, Farley CA. 1998. Survival of infectious Cryptosporidium parvum oocysts in

seawater and Eastern oysters (Crassostrea virginica) in the Chesapeake Bay. Appl Environ Microbiol 64: 1070-1074.
2. Fayer R, Lewis EJ, Trout JM, Graczyk TK, Jenkins MJ, Higgins J, Xiao L, Lal AA. 1999. Cryptosporidium parvum identification in oysters from commercial harvesting sites in the Chesapeake Bay. Emerg Inf Dis 5: 706-710.
3. Graczyk TK, Fayer R, Lewis EJ, Trout JM, Farley CA. 1999. Cryptosporidium oocysts in Bent mussels (Ischadium recurvum) in the Chesapeake Bay. Parasitol Res 85: 518-521.
4. Graczyk TK, Fayer R, Jenkins MC, Trout JM, Higgins J, Lewis EJ, Farley CA. 2000. Susceptibility of the Chesapeake Bay to environmental contamination with Cryptosporidium parvum. Environ Res 78: in press.
5. Graczyk TK, Marcogliese DJ, DaSilva AJ, Mhangami-Ruwende B, Pieniazek NJ. 2000. Cryptosporidium parvum oocysts in Zebra mussels (Dreissena polymorpha) from the St. Lawrence River. Environ Microbiol: in preparation.

Proposed Work Outcome:

GREAT LAKES ZEBRA MUSSELS AS BIOLOGICAL INDICATORS OF WATERBORNE CRYPTOSPORIDIUM CONTAMINATION IN THE VICCINITY OF DRINKING WATERPLANT INLETS

1. A) Applicability of zebra mussels for biological monitoring of waterborne Cryptosporidium contamination, B) Assessment of such contamination in Great Lakes, and C) Determination of the intensity of pathogen load from urban and industrial (wastewater) and agricultural (livestock operation areas) sources to the surface water.
The ability of zebra mussels to recover waterborne C. parvum oocysts was demonstrated recently (1) and the biologic and ecologic features of this species indicate their high applicability for biological monitoring of freshwaters for Cryptosporidium. Zebra mussels would represent a convenient organism for sanitary assessment of water quality because they form dense populations, do not have economic value, are easily collected throughout the year, have a relatively small size, and form clumps that facilitate collection of large samples.

2. A) Assistance to drinking water facility managers.
Under the EPA-implemented regulations, drinking water facilities have to screen raw and finished drinking waters for Cryptosporidium (2). However, Cryptosporidium recovery and detection techniques produce high discrepancy results causing large numbers of water samples to be false-negative (2). Our studies demonstrate that examination of shellfish tissue can prove water pollution with Cryptosporidium even when standard water testing yields negative results (3). Information derived from the examination of zebra mussels collected from the waterplant inlets will be provided to the managers of these facilities who can then cross-examine their Cryptosporidium data derived from water testing.

3. A) Assessment and determination of the important role of populations of Great Lakes zebra mussels in the removal of waterborne C. parvum oocyst load.
A recent study on zebra mussels from the St. Lawrence River, demonstrated that an average of 440 C. parvum oocysts were retained in a single mussel (1). Given the densities reported for this species of 7,000 - 114,000 specimens per square meter and high filtration rate (15 - 122 l/square meter/day, this means that up to 5 x 107 waterborne oocysts can be removed by a square meter of zebra mussel mat. Removal and retention rate of waterborne Cryptosporidium by zebra mussels will be carried out according to our previous protocols (2-3). Ecologic and GIS information on zebra mussel populations will be provided to the project by the Great Lakes Environmental Research Laboratory, NOAA.

Literature Cited

1. Graczyk TK, Marcogliese DJ, DaSilva AJ, Mhangami-Ruwende B, Pieniazek NJ. 2000. Cryptosporidium parvum oocysts in Zebra mussels (Dreissena polymorpha) from the St. Lawrence River. Environ Microbiol: in preparation.
2. Graczyk TK, Cranfield MR, Fayer R. 1997. Recovery of waterborne oocysts of Cryptosporidium parvum from water samples by the membrane-filter dissolution method. Parasitol Res 83: 121-125.
3. Graczyk TK, Fayer R, Lewis EJ, Trout JM, Farley CA. 1999. Cryptosporidium oocysts in Bent mussels (Ischadium recurvum) in the Chesapeake Bay. Parasitol Res 85: 518-521.

Project Milestones:	**Dates:**
Field-collection of zebra mussels	06/2000
Recovery of Cryptosporidium from mussels	10/2000
Cryptosporidium bioinfectivity assays	04/2001
Molecular analysis of Cryptosporidium	10/2001
Data entry, analysis, reports, papers	01/2002
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Project End	05/2002

☐ Project Addresses Environmental Justice

If So, Description of How:

Principal Investigator (PI), researchers and collaborators, involved in the proposed project are acquainted with the environmental justice movement in the US, and prepared to fulfill the requirements as per Title VI of the Civil Rights Act of 1964. The project PI, Dr. Graczyk, will maintain close communication with the USEPA Great Lakes National Program Office, the USEPA Office of Environmental Equity, and the National Environmental Justice Advisory Council. Based on this communication and upon advice from these offices, information on the presence of waterborne Cryptosporidium parvum oocysts and the intensity of waterborne Cryptosporidium contamination in specific regions of Lakes Michigan, Huron, and Erie, will be released (upon request) to the community-based groups in the format requested by these groups. These groups include: A. Community residents and groups, B. Church and civic organizations, C. Business owners, D. Nonprofit organizations, E. Federal, state, tribal, county, and local governments, F. Industry, and G. Educational institutions.

☐ Project Addresses Education/Outreach

If So, Description of How:

1. Communicate scientific and technical information among scientific communities and businesses through press releases, presentations at meetings and publication of results. Any press release or conference will be planned among investigators involved in the project and with the consent of the USEPA's Great Lakes National Program Office.
2. An International Scientific Newsletter "Cryptosporidium Capsule" will be used as a platform for presentation of our results as Dr. Graczyk, serves on the editorial advisory board of this newsletter. "Cryptosporidium Capsule" is a newsletter for government, epidemiology, research, business, and technology news.
3. Basic research on Cryptosporidium and C. parvum strains. We anticipate the possibility of recovering excessive numbers of oocyst isolates of Cryptosporidium species other than C. parvum; these isolates will be provided to specialized laboratories leaded by authorities in Cryptosporidium research in the USA.

Project Budget:

	Federal Share Requested ($)	Applicant's Share ($)
Personnel:	45,080	0
Fringe:	13,098	0
Travel:	2,030	0
Equipment:	0	8,500
Supplies:	20,200	0
Contracts:	20,300	0
Construction:	0	0
Other:	1,015	0
Total Direct Costs:	101,723	8,500
Indirect Costs:	64,594	0
Total:	166,317	8,500
Projected Income:	0	0

Funding by Other Organizations (Names, Amounts, Description of Commitments):

1. The laboratories at John Hopkins University, United States Department of Agriculture (USDA), and Centers for Diseases Control (CDC) (total: approximately 14,500 sq ft) have the necessary facilities and state-of-the-art permanent basic and molecular equipment to carry out the project. Together, we maintain several conventional PCR thermocyclers, automated PCR-gel analysis systems, modern high resolution light/ fluorescent/ phase-contrast/inverted microscope systems, and high-technology systems for testing for waterborne pathogens. Therefore, no costs are anticipated related to the permanent equipment. The permanent equipment present in the PI's (Dr. Graczyk) laboratory alone (value of approximately $45,000) is fully capable of carrying out the activities outlined in the pre-proposal.
2. Researchers from USDA and CDC allocate 5% of their time to the proposed project as in-kind contribution (federal employees are not eligible for financial compensation of their time allocated to the project).

Commitments
Dr. Graczyk: A. project management; scheduling of activities and meetings, communications among personnel, supervision of Cryptosporidium detection, data processing, preparation of publications and reports, and B. Communication with the USEPA Great Lakes National Program Office, USEPA Office of Environmental Equity, and National Environmental Justice Advisory Council to discuss environmental justice issues.

Drinking Waterplant Managers (J. DeKam, R. Johnson, G. Allen) : Assistance in providing zebra mussels from waterplant inlets, and from wastewater discharge and agricultural runoff sites.

USDA (Drs. Fayer, Jenkins, and Higgins) and CDC (Drs. Pieniazek and DaSilva): Assistance with Cryptosporidium bioinfectivity assay, molecular determination of Cryptosporidium species and characterization of C. parvum genotype.

NOAA: Assistance in providing GIS files and zebra mussels from sites not affected by agricultural and industrial pollution.

Description of Collaboration/Community Based Support:

Samples of zebra mussels (3 times/yr, n > 500 mussels) from inlet pipes of drinking water treatment facilities and from sites affected by wastewater discharges and agricultural runoff will be provided by:
1. John A. DeKam, Superintendent. Bay City Water Treatment Plant 2691 N. Euclid Rd., Bay City, MI 48706.
2. Robert Stevenson, Commissioner. City of Toledo, P.O. Box 786, Toledo, OH 43697 (drinking waterplant samples only).
3. Roger C. Johnson, Manager. North Shore Water Commission, 400 W. Bender Rd., Glendale, WI 53217.
4. Gregory Scott Allen. Monroe Water Plant, 915 E. Front St., Monroe, MI 48161.
Waterplant managers will also facilitate contacts with other drinking and wastewater facilities at primary affected areas of concerns.

Zebra mussel samples (3 times/yr, n > 500 mussels) and assistance with GIS files (ARC/INFO, ArcView) will be provided by:
5. Thomas F. Nalepa, Biologist. Great Lakes Environmental Research Laboratory, NOAA, 2205 Commonwealth Blvd., Ann Arbor, MI 48105.
6. Gregory Lang, Biologist, GIS Specialist. Great Lakes Environmental Research Laboratory, NOAA, 2205 Commonwealth Blvd., Ann Arbor, MI 48105.
7. Gregory Gurri-Glass, Associate Professor (GIS expert), Johns Hopkins Univ., SHPH, MMI, Baltimore, MD 21205.
This collaboration will result in maping of urban/industrial (wastewaters) and agricultural (cattle operation areas) sources of Cryptosporidium pollution, and location of drinking water facilities.

Assistance with molecular characterization of Cryptosporidium parvum genotype, i.e., human-or-animal-adapted, will be provided by:
8. Drs. Norman Pieniazek and Alexandre DaSilva, Centers for Diseases Control and Prevention, Division of Parasitic Diseases, Atlanta, GA 30341.

Assistance with Cryptosporidium bioinfectivity assay and molecular determination of Cryptosporidium species will be provided by:
9. Drs. Ronald Fayer, Mark Jenkins, and James Higgins, United States Department of Agriculture, Beltsville, MD 20705.